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Optical detection of asymmetric bacteria utilizing electroorientation

Jae-Woo Choi*, Allen Pu, and Demetri Psaltis

Department of Electrical Engineering, California Institute of Technology, Pasadena, CA 91125

ABSTRACT

We propose a bacterial detection scheme which uses no biochemical markers and can be applied in a Point-of-Care setting. The detection scheme aligns asymmetric bacteria with an electric field and detects the optical scattering.

Keywords: Detection, Electroorientation, Medical optics instrumentation, Optical diagnostics for medicine, Scattering measurements, Urology.

1. INTRODUCTION

The ability to align asymmetric biological particles immersed in a solution of different permittivity with an alternating electric field has been well characterized and applied to the study of several types of elongated cells such as yeast, erythrocytes, bacteria, and algae [1-4]. The ability to measure the orientation and biophysical characteristics of individual bacteria through optical diffraction and scattering techniques has also been well characterized and applied to the study of several species of bacteria [5,6]. In this paper, we demonstrate a bacteria detection scheme that measures the optical scattering from aligned asymmetric bacteria in the presence of an applied alternating electric field by exploiting the fact that most bacteria or bacteria aggregates of interest are asymmetric. We also observed that the alignment occurs only in living bacteria with functional cellular membranes since the cellular membrane becomes permeable when the bacteria die [7].

2. SETUP

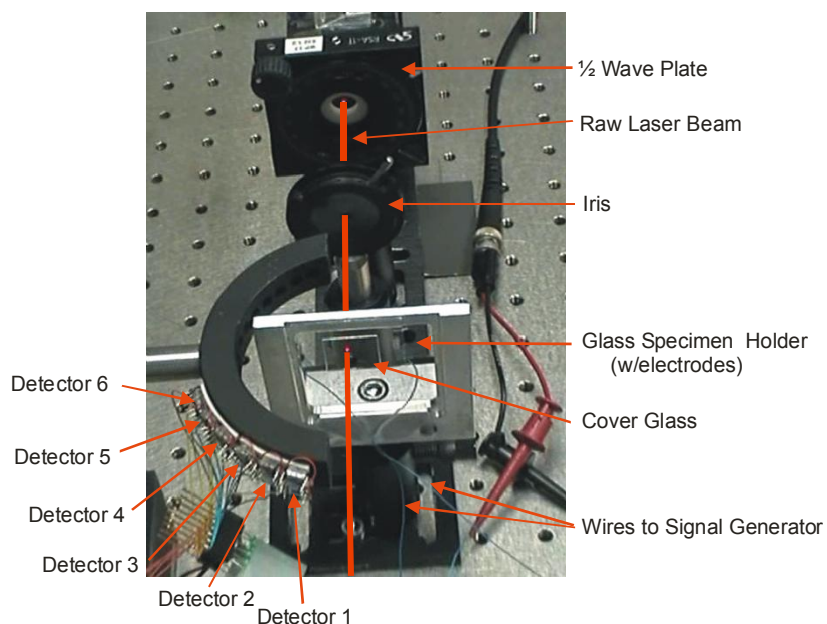


Fig. 1. Diagram of the optical setup.

*choijw@optics.caltech.edu; phone 1 626 395-3889; optics.caltech.edu

The optical setup, shown in Fig. 1, consists of a 5 mW HeNe laser which illuminates a transparent specimen holder consisting of a 1 mm thick glass plate with a conductive electrode pattern in indium tin oxide (ITO) and a thin cover glass, separated by a 20 μm spacer. The void between the glass plates holds approximately 2 μL of test specimen. The laser beam has a diameter of approximately 1.5 mm resulting in an interaction volume of approximately 150 nL. An array of photodiodes at different angles or a simple optical power detector (UDT S370) at a continuous variable angle is used to measure the optical scattering. The specimen holder is connected to a signal generator to provide an alternating voltage of ± 10 V.

An alternating voltage was chosen to eliminate electrolysis in the test specimen at the electrodes. Electrolysis causes bubbles of hydrogen and oxygen gas to form at the boundary of the electrode and liquid. To avoid the creation of bubbles without decreasing the applied voltage, a frequency greater than 1 MHz was necessary. Under observation through the microscope, the asymmetric bacteria aligned to the electric field most efficiently at 10 MHz.

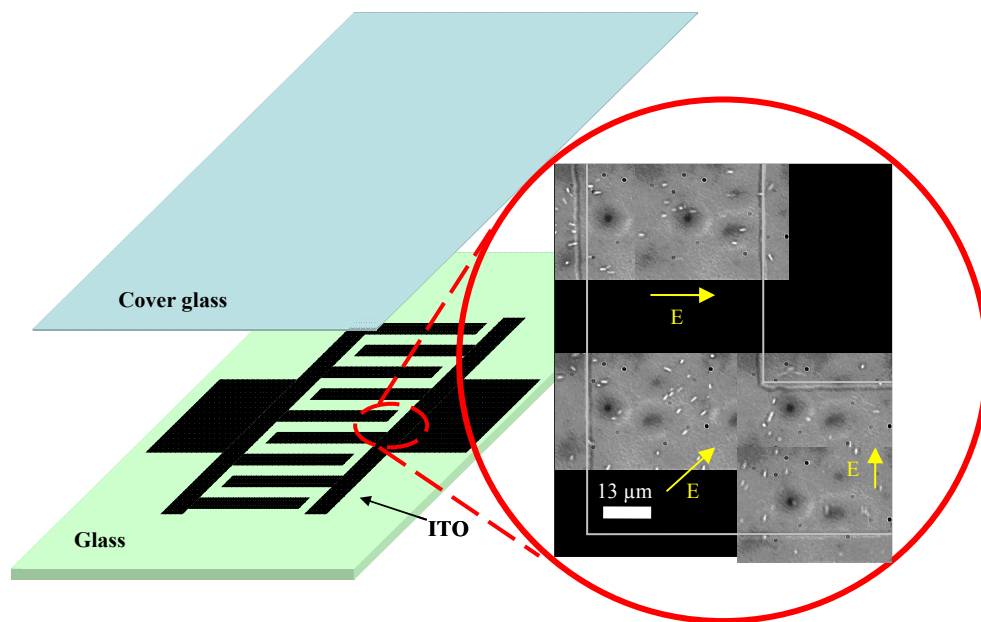


Fig. 2. Schematic diagram of the specimen holder. A 1 mm thick glass plate with a conductive electrode pattern in indium tin oxide (ITO) is separated from a thin cover glass with a spacer. The inset shows a sample of *E. coli* in urine aligned to the applied electric field between the electrodes.

The specimen holder, as shown in Fig. 2, is fabricated using contact photo lithography. First, we obtain ITO coated glass plates (Aldrich) rated at 30-60 Ω/square . Next, positive photoresist (S1813) is spincoated onto the plates. A pattern is exposed using a UV mask aligner and developed. Finally, the ITO is etched to create the electrode pattern.

The conductive electrode pattern is interdigitated as shown in Fig. 2. In determining the optimal electrode spacing and width for optical scattering, there are three factors to consider. First, it has been observed that bacteria do not align over the electrodes. Therefore, the electrode width should be minimized. Second, the spacing between electrodes should be minimized to increase the electric field strength. Third, the electrode width should be maximized to decrease the resistance of the electrode. By testing the performance of different electrode spacing and widths from 10 microns to 500 microns, the optimal electrode spacing and width was determined to be 200 microns and 100 microns, respectively.

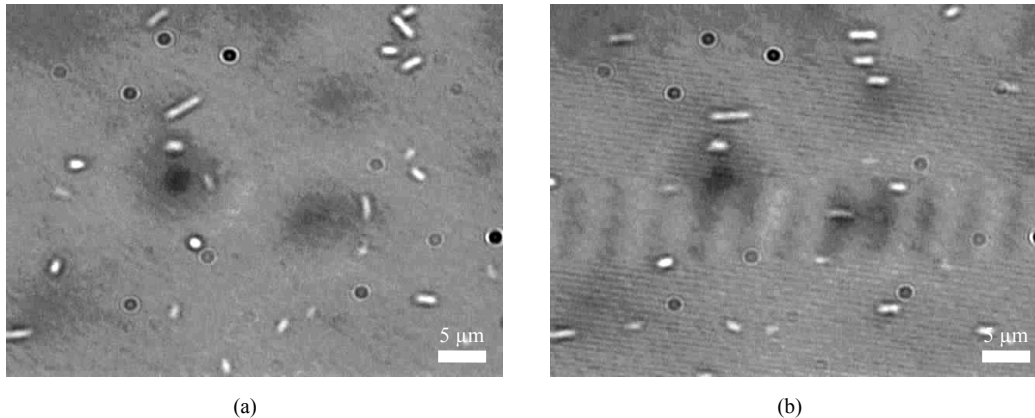


Fig. 3. Live *E. coli* bacteria under 500x magnification. (a) No electric field. The bacteria are aligned randomly. (b) Applied electric field in the horizontal direction. The bacteria are aligned to the electric field. (Online only, 2.45 MB) Initially, the movie shows the bacteria randomly aligned. Then, the electric field is applied and the bacteria align along the field. Note the larger bacteria align slower than the smaller bacteria.

To visualize the effects of the electric field on asymmetric bacteria in solution, the specimen holder is placed under a microscope. Our sample of bacteria is *E. coli* (K12), which are rod-shaped bacteria. When no electric field is applied, live *E. coli* move randomly and are aligned randomly as shown in Fig. 3 (a). When the electric field is applied, live *E. coli* align to the field as shown in Fig. 3 (b); we observe the shorter *E. coli* aligning rapidly and the longer *E. coli* aligning slowly. Even at the corners of the electrodes, *E. coli* align to the field lines, as shown in the inset of Fig. 2. When the electric field is turned off, live *E. coli* move to orient themselves randomly. Note that dead *E. coli* and other symmetric particles do not move or change orientations with respect to the electric field and appear stuck to either the glass plate or cover slide.

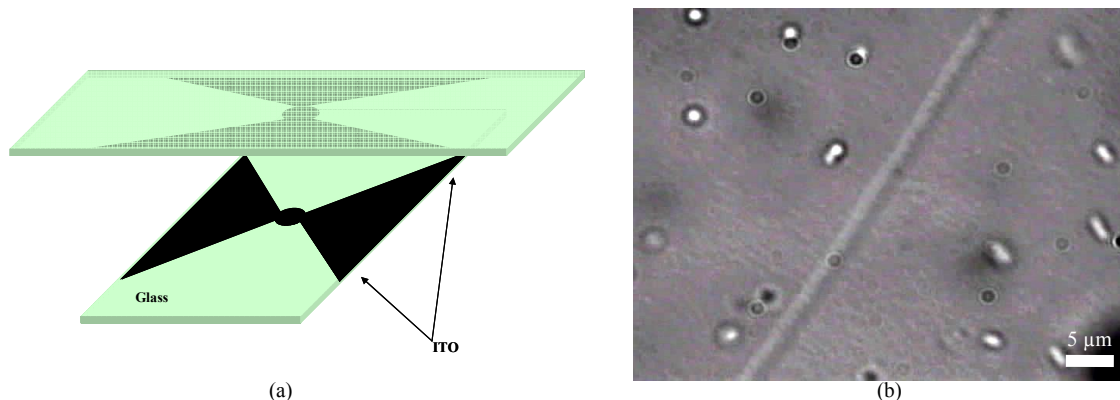


Fig. 4. Specimen holder which aligns bacteria in and out of the plane. (a) Schematic diagram of the parallel plate specimen holder. (b) Applied electric field in and out of the plane. Note the line from the lower left to the upper right of the image denote the boundary of the electrode. Only bacteria to the left of this boundary are aligned to the electric field. Bacteria are randomly orientated to the right of this boundary.

The orientation of the bacteria need not be parallel to the image plane. The orientation of the bacteria may be in and out of the image. By stacking two glass plates with ITO as shown in Fig. 4, we were able to align bacteria perpendicular to the plane of the device. Bacteria that are located just outside of the electrode boundary do not align and remain in random orientations.

3. RESULTS

The ability to detect bacteria through an automated process without expensive and bulky equipment such as an optical microscope would be beneficial for many reasons. First, untrained technicians could operate the equipment. Second, the results may be obtained rapidly. Third, the system could be made small and inexpensive.

To eliminate the need for an imaging system, we have investigated optical scattering as a means to detect the presence of live bacteria. Randomly oriented rod-shaped bacteria have optical scattering measurements with peaks as a function of scattering angle corresponding to the bacteria's radius and length [5,6]. Aligned rod-shaped bacteria have optical scattering measurements with peaks as a function of scattering angle corresponding to either the bacteria's radius or length. In our scheme, the automated detection compares the optical scattering of randomly oriented bacteria versus aligned bacteria.

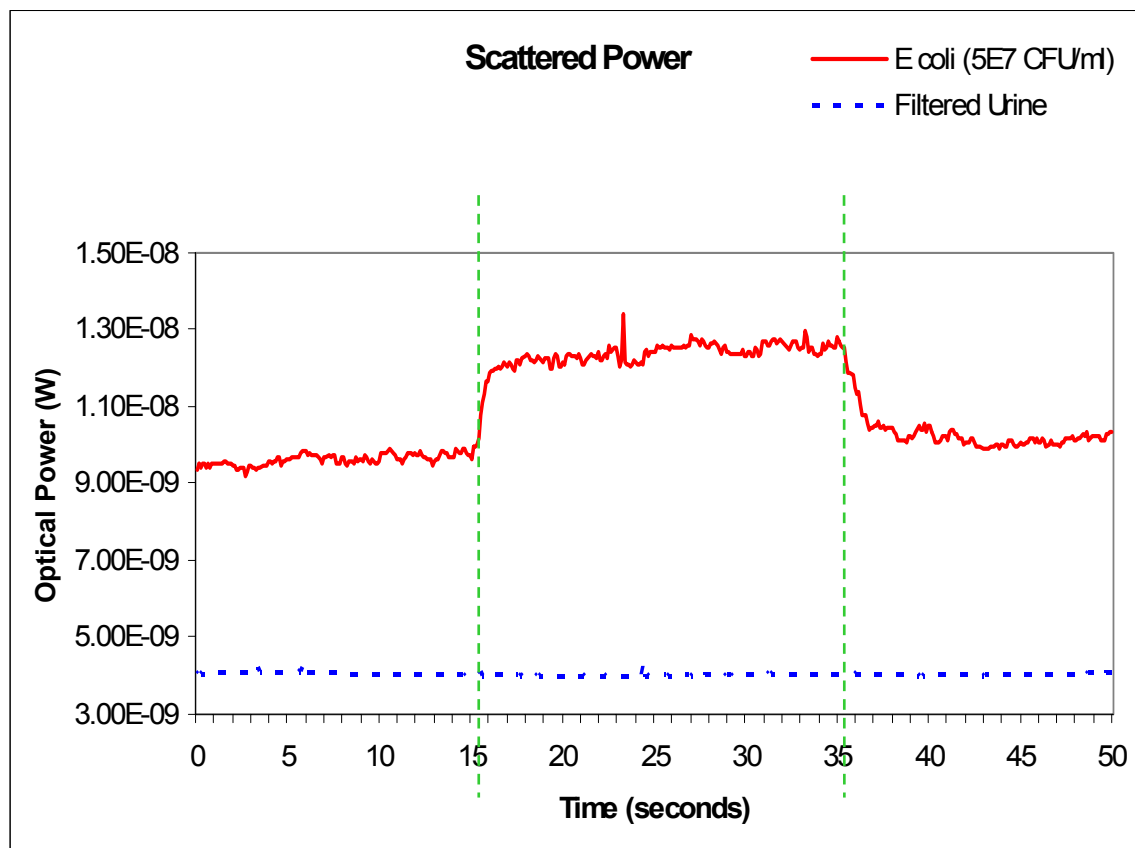


Fig. 5. Optical scattering measurements. The solid curve shows the increase in the optical scattering when the electric field is turned on (between the vertically dashed lines) for $5 \cdot 10^7$ CFU/mL live *E. coli* in urine. The dashed curve shows the same measurement for filtered and sterilized urine with dead *E. coli*.

Fig. 5 shows the optical scattering for samples of $5 \cdot 10^7$ colony forming units (CFU)/mL live *E. coli* in urine and no live *E. coli* in urine. When the electric field is turned on (between the dashed green lines), we notice that the sample of $5 \cdot 10^7$ CFU/mL live *E. coli* in urine has a scattered power increase of 20%. A control experiment with filtered and sterilized urine that contained dead *E. coli* showed no change in the amount of scattered power with an applied electric field.

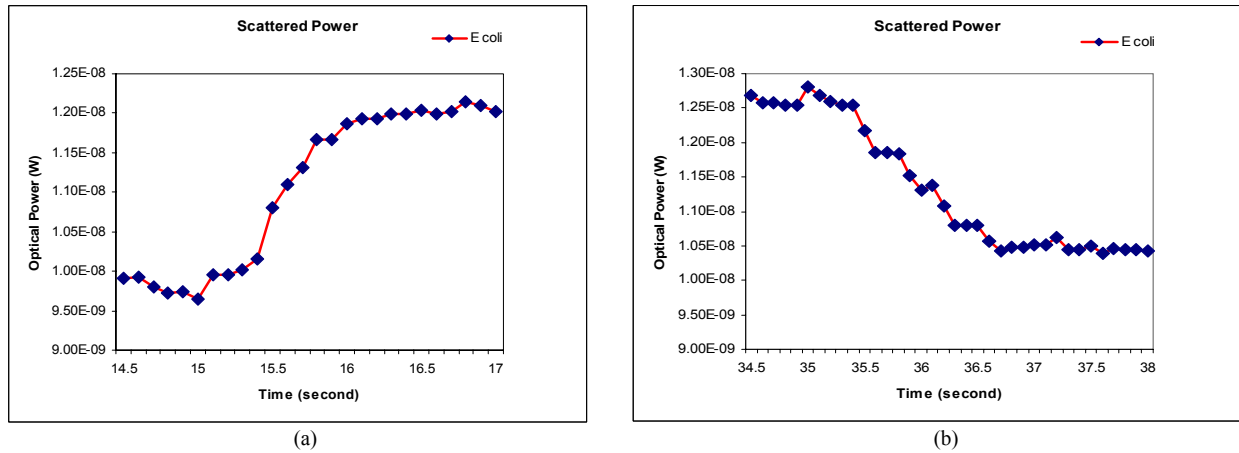


Fig. 6. Rise and fall time of the optical scattering measurement. (a) Rise time of the bacteria alignment to the electric field is approximately 500 milliseconds. (b) Fall time of the bacteria alignment to the electric field is approximately 1.5 seconds.

The optical scattering measurement shows different rise and fall times when the electric field is turned on and off. We noticed that bacteria of different sizes align at different speeds to the electric field. For our particular sample of $5 \cdot 10^7$ CFU/mL live *E. coli*, we have a rise time measurement of approximately 500 milliseconds and a fall time measurement of approximately 1.5 seconds as shown in Fig. 6. The different rise and fall times of bacteria may be due to their different sizes, dielectric constant or polarizability. Therefore, through calibration, we may be able to distinguish the types of different bacteria in our test sample.

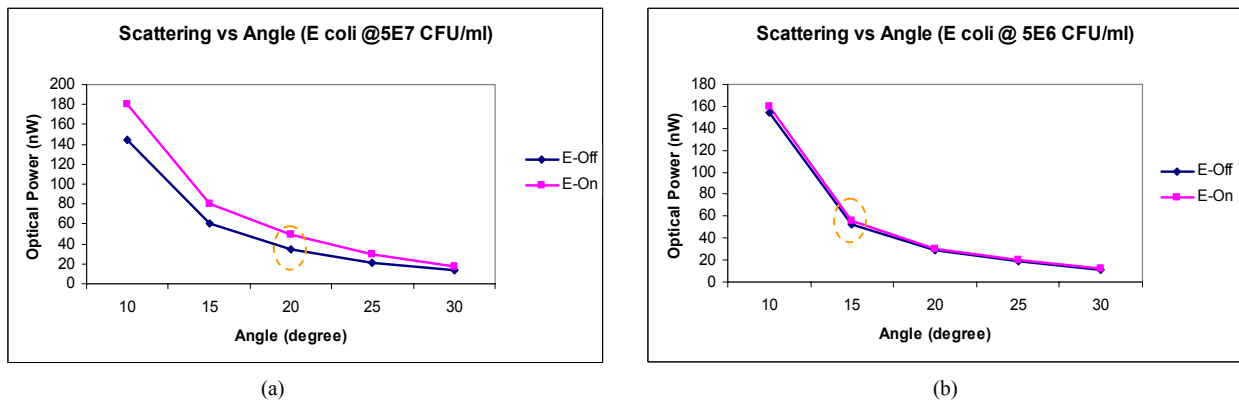


Fig. 7. Optical scattering with respect to bacteria concentration and angle of detector. (a) The measurement with $5 \cdot 10^7$ CFU/mL live *E. coli*. On average, there is approximately a 20% signal increase with the electric field on versus off. At the detector angle of 20 degrees, the signal increase is approximately 44%. (b) The measurement with $5 \cdot 10^6$ CFU/mL live *E. coli*. On average, there is approximately a 5% signal increase with the electric field on versus off. At the detector angle of 15 degrees, the signal increase is approximately 8%.

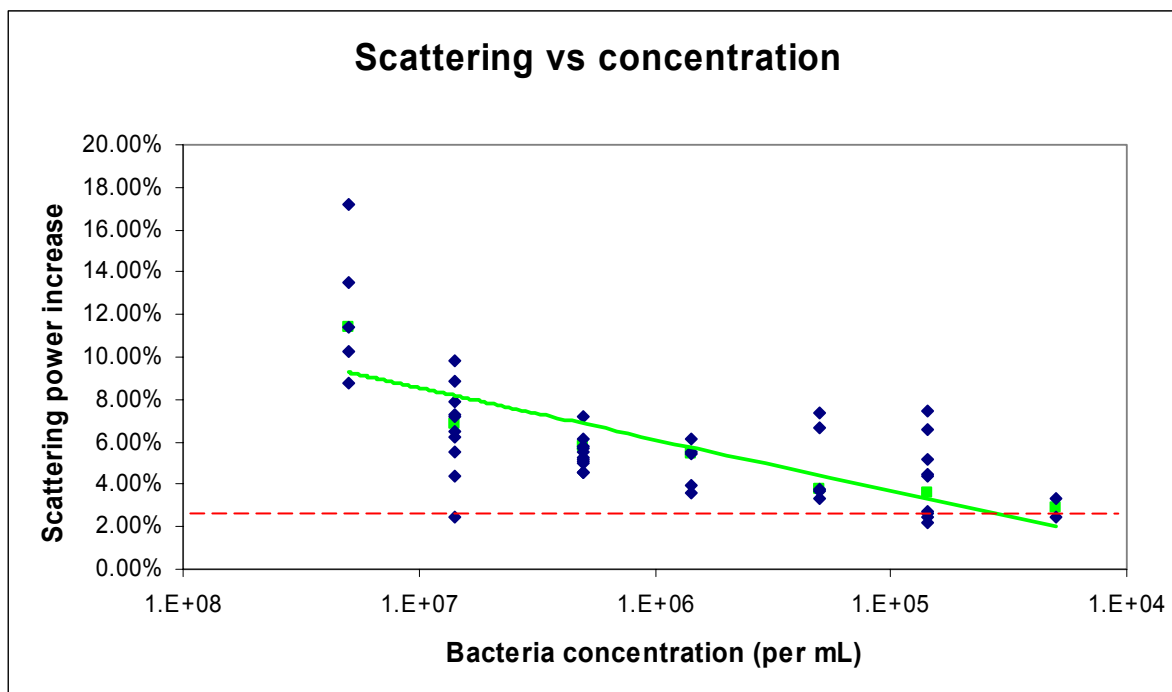


Fig. 8. Optical scattering versus bacteria concentration. Several data points were taken for a concentration of bacteria with each of the scattering power increase measurements shown. The green line is a fitted line through the median of the data points. The red, dashed line indicates the noise floor of urine. Note that the green fitted line reached a threshold at approximately $5 \cdot 10^4$ CFU/mL.

Fig. 7 shows optical scattering measurements with varying bacteria concentration and angle of the detector. Fig. 7 (a) shows similar measurements as shown in Fig. 5. On average, the scattering measurements indicate that there is about a 20% increase when the electric field is turned on for $5 \cdot 10^7$ CFU/mL live *E. coli* in urine. Fig. 7 (b) shows measurement data for $5 \cdot 10^6$ CFU/mL live *E. coli* in urine. There is about a 5% increase when the electric field is turned on. In Fig. 8, these measurements were repeated for several different concentrations multiple times and a line is fitted to the data. The noise floor for urine without bacteria occurs at approximately 1-2%. By comparing our data with this noise floor, we note that the threshold of the optical scattering measurement for detecting bacteria occurs at a concentration which is on the order of magnitude $5 \cdot 10^4$ CFU/mL live *E. coli* in urine.

It has been demonstrated previously that bacteria may be concentrated using a microfluidic circuit that takes advantage of dielectrophoresis (DEP) to redirect live bacteria in to a reservoir [8]. This device has the ability to concentrate live bacteria at four to five orders of magnitude higher than the original concentration. It is fabricated in a similar technique as our device and can be directly integrated with the bacteria detector we describe. Combining the bacteria concentrator with our detection scheme could be useful in diagnosing bacteria infections.

A particularly common bacterial infection is a urinary tract infection (UTI). UTIs result in as many as 7 million visits to outpatient clinics and 1 million visits to emergency departments annually [9]. UTIs are challenging to diagnose due to other diseases that have similar clinical presentation, asymptomatic UTIs, or UTIs with atypical signs and symptoms [10]. The chief culprit of UTIs is *E. coli*, which are rod-shaped bacteria that are diagnosed and treated for by physicians through laboratory examination of urine specimens. In many clinical laboratories, urine cultures are the most common type of culture, accounting for 24%-40% of submitted cultures [11].

Current methods of bacteria detection include urine cultures [12] and biosensor techniques that detect antigen-antibody, enzyme-substrate, or receptor-ligand complexes by measuring fluorescent light, surface reflection, and electrical properties [13-16]. These methods can be time and labor consuming (12-24 hours for urine culture), expensive, or

complex to operate. We have demonstrated a bacteria detection scheme that uses no biochemical markers and has the potential to be small, inexpensive, easy to use for untrained technicians, and provides rapid results.

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